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Microsatellite loci in *Ipomopsis aggregata* (Polemoniaceae) and cross-species applicability for ecological genetics studies[1]

Carrie A. Wu[2,3], Clifford J. Smith[2], and Andrew J. Massaro[2]

[2]Department of Biology, University of Richmond, 28 Westhampton Way, Richmond, Virginia 23173 USA

- *Premise of the study:* Novel microsatellite primers were developed for the native wildflower *Ipomopsis aggregata* to facilitate ongoing studies of the genetics of local adaptation and patterns of hybridization with closely related species within the genus.
- *Methods and Results:* Thirteen primer sets were successfully developed and tested using populations from the Rocky Mountains of Colorado, USA. The primers amplified di-, tri-, and tetranucleotide repeats with 1–15 alleles per locus. All primers also amplified fragments with varying success in closely related *Ipomopsis* species and more distant members of the Polemoniaceae.
- *Conclusions:* The polymorphism levels observed across all loci suggest that these microsatellites may be useful for population genetic studies in *Ipomopsis*, as well as in studies of other related taxa in the Polemoniaceae.

Key words: hybridization; *Ipomopsis*; microsatellite; Polemoniaceae.

The genus *Ipomopsis* Michx. (Polemoniaceae) has long been influential in our understanding of plant speciation and adaptation (Grant and Grant, 1965), and continues to be an important system for investigating the evolution of floral isolation, plant population dynamics, and patterns of selection and gene flow. Hybridization between the closely related species *I. aggregata* (Pursh) V. E. Grant (scarlet gilia) and *I. tenuituba* (Rydb.) V. E. Grant is often extensive throughout the western United States, providing opportunities to explore the processes of local adaptation and speciation. Previous work in one such hybrid zone suggests that the contemporary genetic structure has been influenced by a combination of pollinator and habitat-mediated selection on hybrids and the parental species (reviewed in Campbell, 2005), but elucidating the fine-scale genetic composition of the hybrid populations has proven difficult (Wu and Campbell, 2005). These efforts have largely been challenged by a paucity of genetic markers needed for population genetic studies (Wu, 2006; Stearns et al., 2008). Here, we developed new microsatellite markers for *I. aggregata* to use to explore the genetic structure of natural *Ipomopsis* hybrid populations and in further molecular analyses of the Polemoniaceae.

METHODS AND RESULTS

Microsatellite markers were developed for *I. aggregata* using the protocol of Glenn and Schable (2005). Briefly, genomic DNA extracted from a single plant from Poverty Gulch, Colorado, USA, was digested with *Rsa*I endonuclease and enriched for microsatellite-containing fragments via PCR with biotinylated oligonucleotides. Recovered fragments were cloned using the TOPO TA Cloning system version 2.1 (Invitrogen, Carlsbad, California, USA), and several hundred individual colonies were PCR-amplified and sequenced to screen for fragments that contained microsatellite repeats. For inserts successfully amplified and large enough for sequencing, pairs of forward and reverse primers were designed using Primer3 (Rozen and Skaletsky, 2000) for each putative microsatellite locus identified. PCR amplification of each target microsatellite locus was initially tested using agarose gels, and successful products were verified by Sanger sequencing from two *I. aggregata* individuals. Following this sequence confirmation, an M13R (5′-GGAAACAGCTATGACCAT-3′) or CAG (5′-CAGTCGGGCGTCATCA-3′) sequence tag was added to the 5′ end of one primer of each pair, depending on which would produce the fewest secondary structures as determined with OligoAnalyzer 3.0 (Integrated DNA Technologies, Coralville, Iowa, USA). The addition of the 5′ tag allows use of a third fluorescently labeled primer in the PCR that is complementary to the M13R or CAG sequence tag, reducing the expense for subsequent fragment analysis. Overall, 65 primer pairs were designed from 183 sequenced inserts.

Fragment analysis PCR was performed using Eppendorf Mastercycler thermocyclers with the following conditions: initial denaturation at 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, then a final 7-min extension at 72°C. PCR was conducted in 12.5-μL reaction volumes containing 1.5 mM $MgCl_2$, 0.02 mM of each dNTP, 0.4 μM unlabeled primer, 0.1 μM sequence-labeled primer, 0.4 μM dye-labeled primer (M13R probe with FAM fluorescent dye, CAG probe with HEX), 1.25 U *Taq* (Promega, Madison, Wisconsin, USA) with 1× PCR buffer, and 50 ng DNA. Samples were analyzed at Duke University on an ABI 3730xl sequencer using the ROX 500 size standard following a 1 : 10 dilution, and visualized with GeneMarker software (SoftGenetics, State College, Pennsylvania, USA).

Thirteen of the primer pairs successfully amplified clear products, and were subsequently tested for polymorphism in 12 individuals each from an *I. aggregata* and *I. tenuituba* population in Poverty Gulch, Colorado, USA. Voucher specimens for both species are held at the Rocky Mountain Biological Laboratory Herbarium (*C. F. Williams 9737* and *D. H. Wilken 2921*, respectively). Ten loci were polymorphic in at least one of the two species, with an average of 4.7 ± 4.5 and 4.2 ± 4.4 alleles per locus for the test groups,

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[3]Author for correspondence: cwu@richmond.edu

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TABLE 1. Thirteen microsatellite primers developed in *Ipomopsis aggregata*, and characterized in 12 individuals each of *I. aggregata* and *I. tenuituba* from Poverty Gulch in Colorado, USA.

Primer	Primer sequences (5′–3′)	Repeat motif	Allele size (bp)	*I. aggregata*			*I. tenuituba*			GenBank accession no.
				A	H_o	H_e	*A*	H_o	H_e	
IA14	F: CAGCCTATGAGTGTCCAACTG R: AAGCCCTCAGAAGAACTCCAC	$(CA)_6$	161	3	0.08	0.22	2	0.10	0.10	JQ328028
IA29	F: TCTGTGGTAAGATCGCAATGTC R: AATCACGATAAGCAGCACGTAA	$(GCAT)_3$	253	3	0.08	0.15	1	0	0	JQ328029
IA79	F: CCTACTGGAAAGGATGCAAATC R: CATCAACTGAACAGGCATAGGA	$(CTT)_4$	271	1	0	0	1	0	0	JQ328030
IA102	F: TGCAGGCACAACAGATTCAT R: AAACAAAAGGGCGTCTTTCA	$(TCTA)_{13}$	162	11	0.46	0.69	14	0.80	0.95	JQ328031
IA103	F: CAATCAACTAGCCGTTGGAA R: TGTGAGATGAATCAGTGAAGCTC	$(CAGA)_7$	207	1	0	0	1	0	0	JQ328032
IA324	F: AGAGTGAGAGTGAGGATGAGGA R: AGTCTCTAAAACGCTGAAAAGC	$(AG)_5$	258	1	0	0	1	0	0	JQ328033
IA370	F: TCTAAAGGGAAAAGAGCTTCCA R: GAGGTTTCTCAATGCCACTAGG	$(GAT)_6$	216	4	0.54	0.61	5	0.20	0.44	JQ328034
IA373	F: GGTATTGTCTTTCTCCGACACC R: CCATTTTGCCCTTACTTTGTTC	$(AG)_{10}$	253	10	0.15	0.72	9	0.30	0.89	JQ328035
IA375	F: CTTTCATCTTGCACCTTTTCCT R: GAATTACCATCCCAATCTCCAA	$(CTT)_{13}$	235	2	0.08	0.08	1	0	0	JQ328036
IA387	F: AGGTTGATCCCAAAGCCTCT R: TACCAGACTTCGCCTTTGCT	$(GAA)_{27}$	283	3	0.08	0.22	1	0	0	JQ328037
IA396	F: CCTCGCACACACTCTTCTCA R: GTCCAGATGGAGGAGGATGA	$(AG)_7$	260	5	0.08	0.66	4	0	0.44	JQ328038
IA400	F: GCAGAATCACAGTGAAGGTCAG R: CAGGATATGTTGTCCCAAGC	$(AG)_7$	277	15	0.46	0.81	11	0.50	0.91	JQ328039
IA401	F: CACAACTTCTTAGTGATCCAACTG R: AACTCCACTCAAGCTCCTGAA	$(CA)_{10}$	155	2	0	0.36	3	0	0.59	JQ328040

Note: *A* = number of alleles; H_e = mean expected heterozygosity; H_o = mean observed heterozygosity.

respectively. Analysis with GENEPOP software (Rousset, 2008) indicated that these loci also showed varying levels of heterozygosity (Table 1). Further, following Bonferroni corrections, no polymorphic locus deviated from Hardy–Weinberg equilibrium in either test population, and significant linkage disequilibrium was not detected between any pair of loci in either population. To determine the potential applicability of these primers in related taxa, PCRs were conducted using single individuals from four congeneric species and four more distantly related members of the Polemoniaceae (Porter and Johnson, 2000). Despite the limited sample size for this cross-species screen, each microsatellite locus was successfully amplified in at least two species (Table 2). Further, four of the 13 loci produced amplicons in the most distantly related species tested, *Aliciella pinnatifida* (Nutt. ex A. Gray) J. M. Porter, while each of the remaining species produced successful PCR products with at least seven primer pairs (Table 2). These results indicate the potential of these microsatellite primers for population genetic studies in both *Ipomopsis* and related species.

CONCLUSIONS

The 13 microsatellite primer pairs described here are an important addition to the limited number of genetic markers currently available for *Ipomopsis* (Wu, 2006; Stearns et al., 2008), an emerging system for studying the dynamics of ecological speciation in flowering plants. These polymorphic markers

TABLE 2. PCR amplification success in eight additional members of the Loeselieae tribe within the Polemonieaceae using microsatellite primers developed for *Ipomopsis aggregata*.

Species[a]	IA14	IA29	IA79	IA102	IA103	IA324	IA370	IA373	IA375	IA387	IA396	IA400	IA401
Ipomopsis arizonica Wherry	+	+	+	+	+	+	+	+	+	+	+	+	+
Ipomopsis multiflora (Nutt.) V. E. Grant	+	+	+	+	+	+	+	+	+	+	—	—	+
Ipomopsis sancti-spiritus Wilken & R. Fletcher	+	+	+	+	+	+	+	+	+	+	+	+	+
Ipomopsis tenuifolia (A. Gray) V. E. Grant	+	—	+	+	+	+	+	—	+	—	—	—	+
Eriastrum densifolium (Benth.) H. Mason subsp. *sanctorum* (Milliken) H. Mason	+	—	+	+	+	+	+	—	+	—	—	—	+
Loeselia mexicana Brand	+	+	+	+	+	—	+	—	+	—	—	—	+
Dayia grantii J. M. Porter	+	+	—	+	—	+	+	—	—	+	—	—	+
Aliciella pinnatifida (Nutt. ex A. Gray) J. M. Porter	+	—	—	—	+	—	+	—	—	—	—	—	+

Note: + = successful amplification; — = no amplification.

[a] Species are listed in descending order of relatedness to *I. aggregata*, with the first three species also belonging to the core *Ipomopsis* clade.

should also be useful for population genetic studies within the Polemoniaceae, as evidenced by their amplification success in four congeneric and four more distantly related species.

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